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Taxonomy of the *Gymnopus inusitatus* group and the new *G. inusitatus* var. *cystidiatus* from Hungary

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Abstract — The authors describe a new bisporic basidiomycete, *Gymnopus inusitatus* var. *cystidiatus,* collected in Hungary. It differs morphologically from var. *inusitatus* by the presence of well-developed cheilocystidia and ecologically by growing in the sandy grassland under *Salix*. Its taxonomic position is confirmed by DNA studies.

Key words — *Agaricomycetes*, *Agaricales*, *Omphalotaceae,* phylogeny, ecology

Introduction

During his mycological field research in Hungary, the second author found a fungus macroscopically similar to *Mycetinis scorodonius* (Fr.) A.W. Wilson & Desjardin, but lacking a garlic-like smell. Moreover, microscopic studies showed that it belongs to the genus *Gymnopus* (Pers.) Roussel, species group of *G. inusitatus/bisporus/catalonicus* of sect. *Levipedes* (Fr.) Halling, subsect. *Alkalivirentes* Antonín & Noordel. Species of sect. *Levipedes* are characterized by a pileipellis composed of inflated, often lobed to coralloid, elements (dryophila-type) and the taxa of subsect. *Alkalivirentes* by wall encrustations that turn green in alkali (Antonín & Noordeloos 2010), a division supported by molecular studies. DNA sequences of nuclear ITS and partial LSU ribosomal RNA genes from holotypes of the above-mentioned taxa were identical. Based on this result and morphological similarities, we propose synonymy of *G. inusitatus, G. bisporus,* and *G. catalonicus*. We propose a new variety for the Hungarian collection, which differs microscopically and ecologically from the typical *G. inusitatus*.

Materials & methods

Macroscopic description of collected specimens is based on a photo and dry specimens. Colour abbreviations follow Kornerup & Wanscher (1983); herbarium abbreviations follow Holmgren & Holmgren (1998). Nomenclatural authorities are cited according to the International Plant Names Index Authors website (http://www.ipni.org/ipni/authorsearchpage.do). Microscopic features are described from dried material mounted in H_2O, c. 5% KOH, Melzer's reagent, and Congo red using an Olympus BX-50 light microscope with a magnification of 1000×. Measurements are designated for basidiospores by E (quotient of length and width in any one spore) and Q (mean of E-values) and for lamellae by L (number of entire lamellae) and l (number of series of lamellulae between each pair of entire lamellae).

The nuclear ribosomal RNA ITS and LSU regions from the specimen were sequenced as well as those from the types the type specimens of *Gymnopus bisporus* (BCN–SCM B-4065), *G. catalonicus* (BCN–SCM B-4057), and *G. inusitatus* (BCN–SCM B-4058). Both DNA extraction and PCR amplification were conducted twice, with new PCR reagents used each time.

DNA was isolated from dried herbarium specimens using PowerSoil™ DNA Isolation Kit (Mo-Bio). PCR amplifications were performed in the Mastercycler® ep thermocycler (Eppendorf) according to Tomšovský & Jankovský (2008). *Macrogen* Inc. (Seoul, Korea) sequenced the amplified fragments.

Newly obtained sequences were edited manually using BioEdit version 7.0.9. (T. Hall, Ibis Biosciences). Selected sequences from *Gymnopus* sect. *Levipedes* species. published by Mata et al. (2006) were retrieved from GenBank and added to the dataset (Fig. 1). *Gymnopus brassicolens* and *G. impudicus* were selected as outgroup taxa. The ITS alignment with gaps consisted of 790 characters including 630 constant and 160 variable sites.

Bayesian analysis used MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and best-fit model (GTR+G) and parameters given by MrModeltest (Nylander 2004). Markov chains initiated from a random tree were run for 2,000.000 generations with samples taken every 100th generation. The generation number excluded a burn-in of 200 000 generations; Tracer v1.4.1 (http://tree.bio.ed.ac.uk/software/tracer/) determined the statistics.

The additional PHYML analysis for estimating maximum likelihood phylogenies was run at the server Phylogeny.fr (Dereeper et al. 2008) in "A la Carte" mode. Alignments were treated with Gblock to eliminate poorly aligned positions and ambiguous regions; GTR substitution model was selected for the ITS dataset. Bootstrap branch support values (BP) were estimated in PHYML under the maximum likelihood criterion using default 100 replicates. There were 659 (89% of the original 790 positions) positions analysed in PHYML after Gblock treatment.

The LSU sequences were not analysed due to the limited number of similar sequences available in the Genbank.

Results

Both ITS and LSU sequence analyses showed 100% similarity among the Hungarian collection and the holotypes of *Collybia inusitata*, *C. bispora*, and

Fig. 1. Bayesian phylogram obtained from the ITS rDNA sequences of *Gymnopus inusitatus* and other taxa of *Gymnopus* sect. *Levipedes*. *Gymnopus brassicolens* and *G. impudicus* were selected as outgroup. Bootstrap support values of Maximum likelihood analysis (Phyml) and Bayesian posterior probability values are given at branches. The bar indicates number of substitutions per nucleotide position.

C. catalonica (Fig. 1), indicating that they are conspecific. Both phylogenetic analyses produced almost identical tree topologies. The Bayesian tree had a likelihood value -2192.343. Empirical base frequencies were pi(A) = 0.247, pi(C) = 0.167, pi(G) = 0.210, and pi(T) = 0.377. Model information: parameter α = 0.235; substitution rates A–C = 0.062, A–G = 0,495, A–T = 0.070, C–G = 0.012, C–T = 0.286, G–T = 0.073; tree-length = 0.488.

Fig. 2. *Gymnopus inusitatus* var. *cystidiatus* (holotype). Scale bar = 10 mm. Photo P. Finy.

Taxonomy

Microscopical examination of the types and Hungarian collection noted above confirmed the synonymy indicated by the sequence analyses. We propose the following taxonomy for this species group of bisporic taxa:

Gymnopus inusitatus (Vila & Llimona) Vila & Llimona, Rev. Catal. Micol. 28: 180. 2006.
≡ *Collybia inusitata* Vila & Llimona, Rev. Catal. Micol. 24: 84. 2002.
= *Collybia bispora* J. Carbó & Pérez-De-Greg., Rev. Catal. Micol. 24: 278. 2002.
≡ *Gymnopus bisporus* (J. Carbó & Pérez-De-Greg.) Vila & Llimona, Rev. Catal. Micol. 28: 180. 2006.
= *Collybia catalonica* Vila & Llimona, Rev. Catal. Micol. 24: 80. 2002.
≡ *Gymnopus catalonicus* (Vila & Llimona) Vila & Llimona, Rev. Catal. Micol. 28: 180. 2006.

Gymnopus inusitatus* var. *cystidiatus Antonín, Finy & Tomšovský, **var. nov.**
MycoBank MB 563276 Figs 2–3

A varietate typica cheilocystidiis presentibus et oecologiae (sub Salice*) differt.*

Type: Hungary, Székesfehervár–Sóstó, 14.IX.2010, leg. P. Finy (**Holotype**, BRNM 737257; GenBank JN247550, JN247554).

Etymology: having well-developed cheilocystidia.

Basidiomes similar to *Mycetinis scorodonius*. Pileus up to 10 mm broad, convex to applanate, with plane to depressed centre, smooth, slightly crenulate

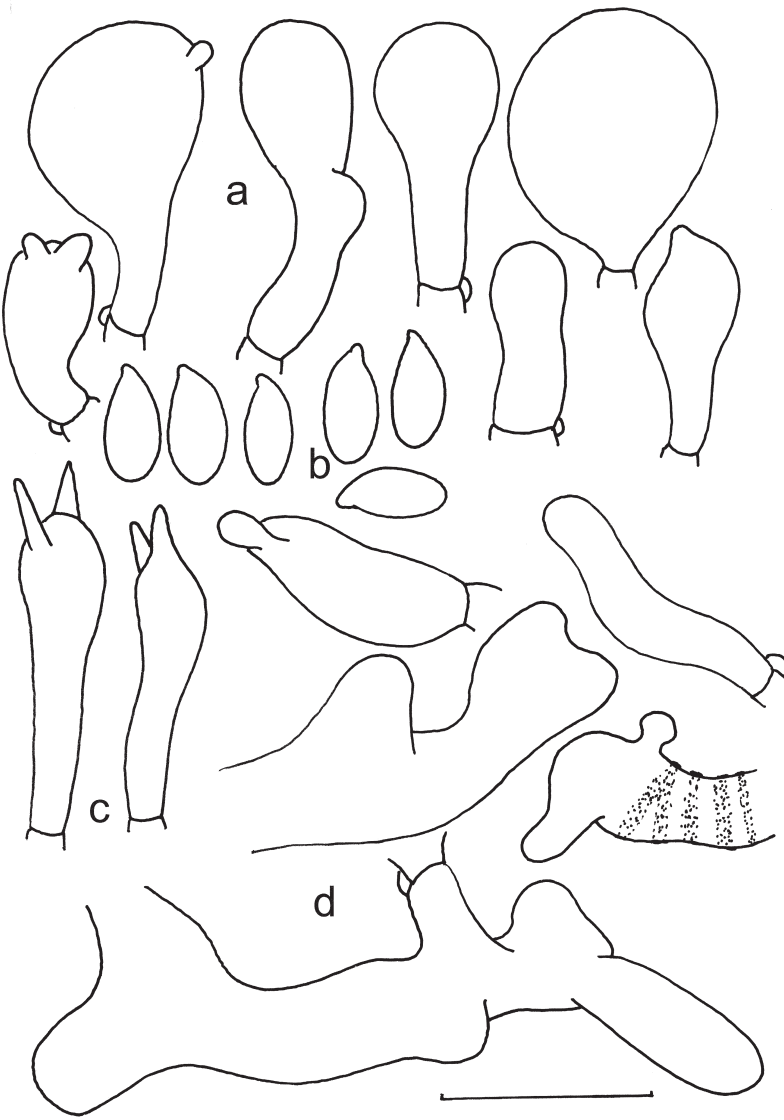

Fig. 3. *Gymnopus inusitatus* var. *cystidiatus* (holotype).
a. cheilocystidia, b. basidiospores, c. basidia, d. pileipellis. Scale bar = 20 μm.

at margin especially when old, brown to reddish brown (7–8E7) at centre, paler towards margin. Lamellae rather distant, L = 14–17, l = 2–3, emarginate and attached with tooth, ± ventricose, whitish, with concolorous edge. Stipe up to 35 × 0.75 mm (dry specimens), cylindrical, slightly broadened at apex, cylindrical to slightly clavate at base, smooth, ± concolorous with pileus at base with paler upper part; basal mycelium brown to reddish brown. Context without any distinct smell; taste not tested.

Basidiospores (10–)10.5–13 × 4.5–6.0 µm, average = 11.3 × 5.3 µm, E = 1.9–2.7, Q = 2.15 (40 spores, 2 basidiomes, 1 collection), ellipsoid-fusoid, pip-shaped, thin-walled. Basidia 26–33 × 7.5–8.5 µm, 2-spored, clavate. Basidioles up to 43 × 4.0–9.0 µm, cylindrical, clavate, subfusoid. Cheilocystidia 18–34(–40) × 7.0–12(–20) µm, clavate, subutriform, often capitate, sometimes irregular, smooth or with projection(s), thin-walled. Trama hyphae ± cylindrical, smooth or minutely incrusted, up to 12 µm wide. Pileipellis a cutis composed of cylindrical to slightly inflated elements, sometimes irregular or with projections, ± thin-walled, coarsely incrusted (zebroid), 3.0–10 µm wide hyphae with greenish brown incrustation in KOH; terminal cells cylindrical, clavate, fusoid, only minutely incrusted or smooth, often irregular or with projections; some elements transient to poorly developed dryophila-structure. Stipitipellis a cutis of parallel, cylindrical, slightly thick-walled, incrusted, up to 5.0 µm wide hyphae. Caulocystidia absent, scattered appressed to (sub)erect, cylindrical, rare terminal cells with scattered projections present. Clamp connections present.

Chemical reactions — No part of basidiocarps amyloid or dextrinoid.

Ecology & distribution— Between *Salix* and grass remnants in open sand calcareous dune near *S. cinerea* and *S. purpurea*. So far known only from the type locality.

Additional specimens examined — ***Gymnopus bisporus***: Spain, Catalonia, Girona, La Fonollera, Torroella de Mongrí, 1 Oct. 2000, J. Carbó (holotype of *Collybia bispora*, BCN–SCM B-4065, GenBank: JN247551, JN247555).

Gymnopus catalonicus: Spain, Catalonia, Barcelona, Can Ferrer, 23 Oct. 2000, J. Vila & X. Llimona (holotype of *Collybia catalonica*, BCN–SCM B-4057), GenBank: JN247552, JN247556.

Gymnopus inusitatus: Spain, Catalonia, Roses, Coll de la Perafita, 6 Oct. 1998, J. Vila & X. Llimona (holotype of *Collybia inusitata*, BCN–SCM B-4058), GenBank: JN247553, JN247557.

Discussion

Gymnopus inusitatus var. *cystidiatus* is characterized macroscopically (Fig. 2) by having basidiomes similar to *Mycetinis scorodonius*, and microscopically (Fig. 3) by rather large basidiospores, long, 2-spored basidia, well-developed clavate, subutriform, often capitate cheilocystidia, a pileipellis composed of

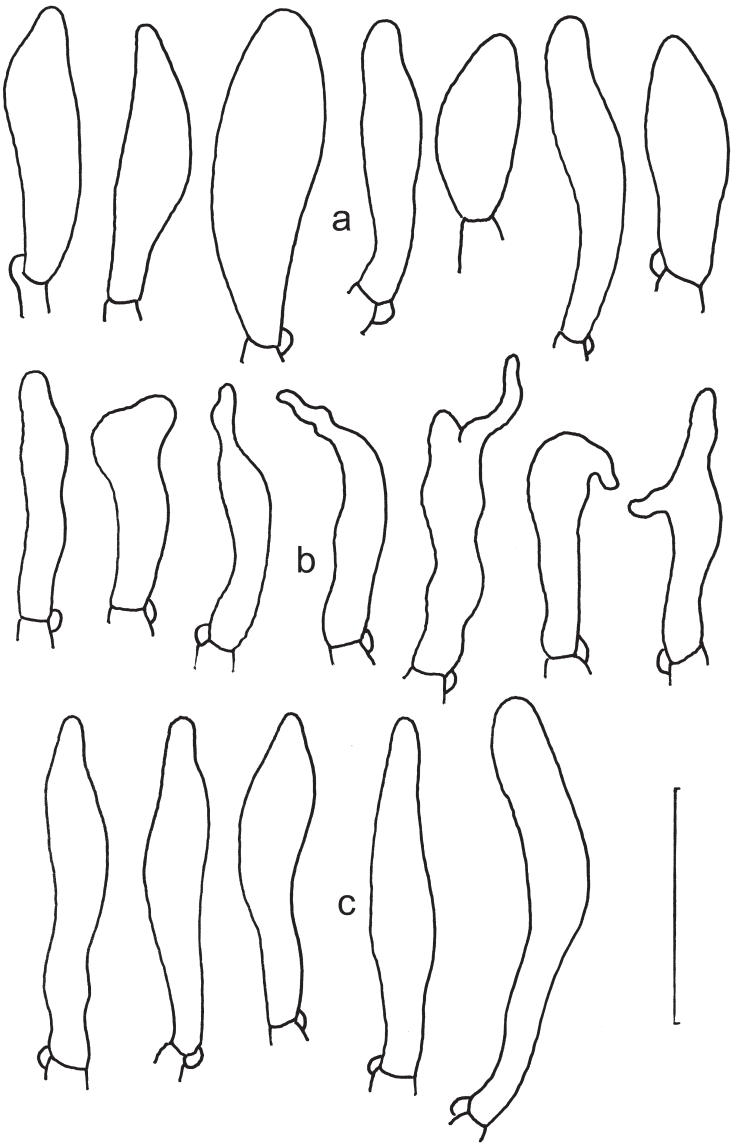

Fig. 4. *Gymnopus inusitatus* var. *inusitatus*. Marginal cells of lamellae (holotypes). a. *Collybia inusitata*, b. *C. bispora* and c. *C. catalonica*). Scale bar = 20 μm.

hyphae with greenish brown incrustation in KOH forming a cutis or only a poorly developed dryophila-structure, and cylindrical, clavate, fusoid, often irregular terminal cells, an absence of caulocystidia, and ecologically by growing in sandy grasslands under *Salix*.

In this group of bisporic taxa, Antonín & Noordeloos (2010) distinguished two species — *Gymnopus bisporus* (syn. *G. catalonicus*) with a brown to reddish brown (marginally paler) pileus and basidiospores measuring 9.0–11(–12) × 4.5–5.5(–6.0) µm and *G. inusitatus* with a reddish brown to violaceous brown pileus (cream to ochraceous-brown when drying), and slightly narrower basidiospores measuring 10–14 × 4.0–5.0 µm. Both species lack true cheilocystidia (Fig. 4). The basidiospore size variation in this group of taxa is distinctly wider than admitted by the current anatomic-morphological species concepts. The same situation was noted for European species in the *Psilocybe cyanescens* group (Borovička 2008, Borovička et al. 2010).

The most important diagnostic characters of *G. inusitatus* var. *cystidiatus* are the presence of well-developed cheilocystidia and a different. All three taxa synonymized under of *G. inusitatus* var. *inusitatus* were collected in xerothermic acidophilous stands with various *Cistus* species (Pérez-De-Gregorio & Carbó 2002, Vila & Llimona 2002). *Gymnopous inusitatus* var. *cystidiatus* was collected in calcareous sandy grasslands under *Salix*. Its type locality lies in the Great Hungarian Plain in the northwestern part of the Pannonic sand steppes where the open xeric grassland is dominated by the Pannonian endemic *Festuca vaginata* on dune tops and communities of *Salix rosmarinifolia* and other *Salix* species in the moist interdune depressions. The Hungarian collection represents the first record of *G. inusitatus* in central Europe.

Species from the *G. dryophilus* group differ from *G. inusitatus* by a well-developed dryophila-structure in the pileipellis, tetrasporic basidia, and smaller basidiospores. *Gymnopus erythropus* (Pers.) Antonín, Halling & Noordel. has red-brown coloured hairs on the stipe base like *G. inusitatus* but differs in basidiome colour, smaller basidiospores ((5.0–)5.5–8.0(–9.0) × 3.5–4.5(–5.0) µm), tetrasporic basidia, and differently shaped cheilocystidia (Antonín & Noordeloos 2010).

The macroscopically similar *Gymnopus earlei* Murrill from North America has a larger (≤ 35 mm broad) pileus, buff or pale orangish yellow (later orangish buff) lamellae, a more robust stipe (10–46(–90) × 2–5 mm), smaller basidiospores (5.6–7 × 2.8–3.5 µm), mostly tetrasporic basidia, and inconspicuous cheilocystidia (Halling 1983, Vilgalys & Miller 1983). *Gymnopus bicolor* A.W. Wilson et al. has similar cheilocystidia and a poorly developed dryophila-structure, but it has crowded lamellae, a larger (22–28 mm broad) pileus, a more robust stipe (30–42 × 2–3 mm), tetrasporic basidia, and smaller basidiospores (5.2–8 × 2.4–3.6 µm; Wilson et al. 2004).

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